

C2
Figure 2. Structure and sequence of full length rat OPG gene, a novel member of the TNFR superfamily. A. Map of pMOB-B1.1 insert. Box indicates position of LORF within the cDNA sequence (bold line). Black box indicates signal peptide, and gray ellipses indicate position of cysteine-rich repeat sequences. B, C. (SEQ ID NOS: 120 & 121) Nucleic acid and protein sequence of the Rat OPG cDNA. The predicted signal peptide is underlined, and potential sites of N-linked glycosylation are indicated in bold, underlined letters. D, E. (SEQ ID NOS: 128, 129, 130, 131, 132, 133, 234, 135 & 136) Pileup sequence comparison (Wisconsin GCG Package, Version 8.1) of OPG with other members of the TNFR superfamily, fas (SEQ ID NO:128); tnfr1 (SEQ ID NO: 129); sfu-t2 (SEQ ID NO:130); tnfr2 (SEQ ID NO:131); cd40 (SEQ ID NO:132); osteo (SEQ ID NO:133); ngfr (SEQ ID NO:134); ox40 (SEQ ID NO:135); 41bb (SEQ ID NO:136).

At page 7, replace the last paragraph, lines 1-2 and page 8, first paragraph, lines 1-5, with the following:

C3
Figure 9. Structure and sequence of mouse and human OPG cDNA clones. A, B. (SEQ ID NOS: 122 & 123) Mouse cDNA and protein sequence. C, D. (SEQ ID NOS: 124 & 125) Human cDNA and protein sequence. The predicted signal peptides are underlined, and potential sites of N-linked glycosylation are indicated in bold. E, F. (SEQ ID NOS: 121, 123 & 125) Sequence alignment and comparison of rat, mouse and human OPG amino acid sequences.

At page 8, replace the second paragraph, lines 7-14, with the following:

C4
Figure 10. (SEQ ID NOS: 126 & 172) Comparison of conserved sequences in extracellular domain of TNFR-1 and human OPG. PrettyPlot (Wisconsin GCG Package, Version 8.1) of the TNFR1 and OPG alignment described in example 6. Top line, human TNFR1 sequences encoding domains 1-4. Bottom line, human OPG sequences encoding domains 1-4. Conserved residues are highlighted by rectangular boxes.

At page 8, replace the last paragraph, lines 29-36 and page 9, first paragraph, lines 1-8, with the following:

C5
Figure 12. (SEQ ID NOS: 138 & 139) Structure of OPG cysteine-rich domains. Alignment of the human (top line) and mouse (bottom line) OPG amino acid sequences highlighting the predicted domain structure of OPG. The polypeptide is divided into two halves; the N-terminus (A), and C-terminus (B). The N-terminal half is predicted to contain four cysteine rich domains (labeled 1-4). The predicted intrachain disulfide bonds are indicated by bold

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lines, labeled "SS1", "SS2", or "SS3". Tyrosine 28 and histidine 75 (underlined) are predicted to form an ionic interaction. Those amino acids predicted to interact with an OPG ligand are indicated by bold dots above the appropriate residue. The cysteine residues located in the C-terminal half of OPG are indicated by rectangular boxes.

At page 9, replace the last paragraph, lines 27-36 and page 10, first paragraph, lines 1-6, with the following:

C6

Figure 14. Expression of human OPG in E. coli. A. (SEQ ID NOS: 127 & 137) Construction of a bacterial expression vector. The LORF of the human OPG gene was amplified by PCR, then joined to a oligonucleotide linker fragment (top strand is SEQ ID NO:137; bottom strand is SEQ ID NO:127), and ligated into pAMG21 vector DNA. The resulting vector is capable of expressing OPG residues 32-401 linked to a N-terminal methionine residue. B SDS-PAGE analysis of uninduced and induced bacterial harboring the pAMG21-human OPG -32-401 plasmid. Lane 1, MW standards; lane 2, uninduced bacteria; lane 3, 30°C induction; lane 4, 37°C induction; lane 5, whole cell lysate from 37°C induction; lane 6, soluble fraction of whole cell lysate; lane 7, insoluble fraction of whole cell lysate; lane 8, purified inclusion bodies obtained from whole cell lysate.

At page 10, replace the last paragraph, lines 20-35, with the following:

C7

Figure 16. Pulse-chase analysis of recombinant murine OPG produced in CHO cells. CHO cells were pulse-labeled with ³⁵S-methionine/cysteine, then chased for the indicated time. Metabolically labeled cultures were separated into both conditioned media and cells, and detergent extracts were prepared from each, clarified, then immunoprecipitated with anti-OPG antibodies. The immunoprecipitates were resolved by SDS-PAGE, and exposed to film. Top left and right panels (A); samples analyzed under non-reducing conditions. Lower left and right panels (B); samples analyzed under reducing conditions. Top and bottom left panels; Cell extracts. Top and bottom right panels; Conditioned media extracts. The relative mobility of the 55 kd monomeric and 100 kd dimeric forms of OPG are indicated by arrowheads.

At page 12, replace the third paragraph, lines 17-26, with the following:

C8

Figure 23. Effects of OPG on calvarial osteoclasts in control and IL1-treated mice. Histological methods for analyzing mice calvarial bone samples are described in Example 11B. Arrows indicate osteoclasts present in day 2-treated mice. Calvarial samples of mice receiving four PBS injections daily (A), one injection of IL-1 and three injections of PBS daily (B), one injection of PBS and three injections of OPG daily (C), one injection of IL-1 and three injections of OPG daily (D).